Disinfection with BPL

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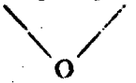
THE search for liquids and vapors for sterilizing enclosed areas contaminated with microorganisms prompted investigation of the efficacy of vaporized beta-propiolactone. Beta-propiolactone* (BPL) is an alkylating agent with an empirical formula $CH_2.CH_2.C{=}O$, similar in



structure to ethylene oxide. It is a colorless liquid at room temperature, boils at 162.3°C., has a specific gravity of 1.1490, (1) and a vapor pressure of about 3.4 millimeters of mercury at 25°C. (2) The vapor is nonflammable and noncorrosive at the recommended concentration. Commercial grade BPL (97 per cent) is stable for several years when stored at 4°C., (2) and the half-life of aqueous solutions is approximately three and one-half hours at 25°C. (3) These characteristics, in addition to its known bactericidal and sporicidal action, justified the evaluation of BPL vapor as a sterilant for aerosol vessels.

The sterilization efficacy of BPL vapor is a direct function of concentration, relative humidity, and temperature. A temperature of 25°C., or higher, is desirable; the effectiveness of the disinfectant decreases rapidly at relative humidities below 70 per cent.

Although BPL has several disadvantages (toxicity, solvent action), it is in several respects superior to most other sterilants. Among the advantages of BPL are: (a) it has good bactericidal properties, (b) it acts rapidly, (c) its residual end-products are easily neutralized and are readily soluble in water, and (d) its vapors are lachrymatory (it thereby serves as its own warning device), but are less irritating than those of other sterilants.

The aerosol chambers used in these tests cannot be sealed to permit sterilization with steam under pressure, ethylene oxide gas mixtures, or other procedures requiring pressure or vacuum. Use of highly corrosive chemical sprays such as peracetic acid are not normally feasible because of deleterious effects on the materials of the chambers. Until May 1956, these chambers were sterilized routinely by admitting formaldehyde in the presence of large amounts of steam. For safety reasons, the vessels were maintained at a slightly reduced air pressure (1.0 inch of water) during sterilization. Although formaldehyde has proved satisfactory, in many cases its use imposed serious operational limitations. These limitations can be summarized:

(a) Formaldehyde tends to deposit solid polymers (e.g. paraformaldehyde) that are difficult to neutralize and remove after the sterilization process. These polymers slowly revert to formaldehyde gas, thus creating a problem of aeration. It is necessary to steam and air-wash the chambers for one or two 2-hour periods followed by an air-wash of 24 to 48 hours' duration to remove the polymerization products and to prevent subsequent release of formaldehyde vapors.

(b) Even when the formaldehyde vapor concentration is reduced below the detectable limits of the chromotropic acid assay, there still may be a residual effect that will affect the validity of aerosol data.

(c) The toxicity of formaldehyde vapors and polymers to human tissue creates a problem, because it is often necessary for workers to enter the chambers after sterilization to work or equipment or remove pieces of equipment for repair.

Test Procedure

The microorganisms used in the evaluation of BPL were aerosolized in three test chambers with capacities of 50,000 liters, 100,000 liters, and 1,000,000 liters.

Serratia marcescens and spores of *Bacillus subtilis* var. *niger* and *Bacillus anthracis* were used as test organisms. The volume of test material aerosolized in the smaller chambers varied from 10 to 35 milliliters of a suspension containing 2×10^{10} organisms per milliliter. In the 1,000,000-liter chamber, 100 milliliters of a suspension containing 2×10^{11} organisms per milliliter was used.

The same basic pattern was

Beta-propiolactone found rapid and effective sterilant for microbiologically contaminated aerosol chambers, provided temperature, time, humidity, concentration are correctly adjusted

*Source: Calcium Corp. of America, New York, 16.

followed in all of the sterilization tests. The relative humidity (RH) of the aerosol chamber was adjusted prior to the dissemination of the BPL to obtain a final RH of 80 to 85 per cent. Incomplete disinfection results if the RH is below 70 per cent; if the RH is too high condensation occurs, causing fallout of the beta-propiolactone onto the surfaces. Approximately 15 minutes prior to sterilization the BPL to be disseminated was mixed with water in a 1:3 ratio.

Preliminary tests indicated that three milligrams of BPL per liter of air did not sterilize the test chambers; therefore, the amount of BPL was increased to give an air concentration of approximately six milligrams per liter. The concentration of BPL vapor in the air was measured by withdrawing air samples and analyzing the samples spectrometrically

After a contact period of 30 minutes, an additional 3 milligrams of BPL per liter of chamber volume was disseminated to compensate for fallout (as condensation) of the vapor and to maintain a theoretical concentration of six milligrams of BPL per liter of air. (See appendix B for disseminating devices and sources). A final contact period of one hour was maintained (total contact time, one and one-half hours). The inside of the 1,000,000-liter chamber was then washed with 275 gallons of 0.5 per cent sodium thiosulfate solution to neutralize the BPL. The neutralization treatment was followed by a water rinse to remove residual thiosulfate and other residual products. Further tests have shown that no thiosulfate wash is necessary and that water alone is entirely satisfactory.

Following the BPL contact period and subsequent water rinse, the viable test microorganisms were detected by sampling the interior surfaces of the chambers with sterile cotton swabs that had been immersed in physiological saline. The cotton swab samples were streaked on media suitable for the recovery of the microorganisms under test:

Bacillus anthracis—five per cent sheep's blood agar;

Bacillus subtilis var. *niger*—nutrient agar; and

Serratia marcescens—nutrient agar containing 0.5 per cent Tamol "N".*

All plate samples were read after 24 and 48 hours incubation at optimum temperature.

Test Results

The interiors of the chambers were purposely contaminated with vegetative cells of *Serratia marcescens*, or spores of either *Bacillus anthracis* or *Bacillus subtilis* var. *niger*. A total of 39 sterilization trials were conducted in the three chambers. (Table 1):

Of the 2850 surface samples taken following these sterilization tests, only one sample from each of two different trials was positive for the test organisms. The failure to achieve sterilization in these two trials was attributed to faulty dispersion of the BPL caused by (a) excessively long dissemination time, and (b) the solvent action of BPL on the painted inner surface of the disseminating device. These failures are indicative of the need for dispersing the BPL quickly throughout the spaces to be sterilized. (see appendix B) Rapid dissemination of BPL is required to compensate for the accelerated decay of BPL when aerosolized as small droplets which collectively represent a large surface area.

Discussion

Before BPL was employed to sterilize chambers, other sterilants were used effectively, but with undesirable limitations. These were imposed by the chambers themselves, the corrosive effect of chemical sprays, the formation of polymers requiring lengthy neutralization procedures, cleansing problems of the interior surfaces of the chambers, residual traces that could affect the validity of subsequent experiments, and toxicity to humans.

Residual BPL

The primary advantage of BPL as a sterilant in the chambers is its ease of removal after the sterilization cycle. In the tests described here, residual BPL was neutralized by washing the inside of the chamber with 0.5 per cent sodium thiosulfate solution. Subsequently, a series of measurements to determine residual BPL after treatment indicated that the thiosulfate wash was not necessary. Quantitative assay for residual BPL following treatment without a thiosulfate wash indicated a residuum of less than one gamma per liter of air within one-half hour after a cold water wash. Of more critical importance than the chemical assay is the biological effect of using fastidious vegetative microorganisms. To date, no biological interference has been detected between tests in which the thiosulfate was used and those in which it was omitted.

Corrosion Problems

The use of any sterilant in chambers raises questions of corrosive action on metal fittings and damage to rubber products such as gloves and gaskets. Corrosion of metal products by BPL is negligible, (3) but occasionally galvanic corrosion may occur as in any bimetallic system. BPL may have a deleterious effect on neoprene products if it is not removed with water immediately after the contact period.

Personnel Protection

BPL is toxic not only upon inhalation, but can also cause erythema and vesication if a high concentration of its vapor, or the liquid, remains in contact with the skin for an extended time. The vapor of BPL in low concentrations is irritating and lachrymatory. (1) Severe skin burns have re-

*Tamol "N" leaches the red pigment from the colony into the surrounding agar for rapid identification.

Table 1.

Chamber capacity Liters	Microorganisms B. anthracis	S. marcescens	B. subtilis
50,000	5	16	0
100,000	5	7	2
1,000,000	0	1	3[a]

a. In each of two of these tests viable cells were recovered on one cotton swab.

sulted from spills of liquid BPL on shoes and clothing. The incorporation of brilliant green dye as one part per million in the BPL-water mixture can aid visual detection of BPL on skin and clothing. The presence of the dye on clothing indicates the need for an immediate clothing change and shower to prevent skin burns. The incorporation of the dye in the BPL-water mixture does not lessen its effectiveness, as a sterilant. When handling BPL, wearing of protective clothing, including rubber boots, gloves, apron, and face shield is recommended.

BPL as a vapor may be detected by the gas detection tubes developed by the Chemical Corps, which were recently incorporated in Civil Defense survival kits. (4) The sensitivity of the detection tubes is 0.002 milligram of BPL. This test is based on the blue color caused by the alkylation of gamma (p-nitrobenzyl) pyridine adsorbed on silica gel inside a small glass tube through which air is drawn by a hand pump. Similar detection tubes are also available commercially.*

Conclusions

The tests described indicate that beta-propiolactone is an excellent sterilant for the air and surfaces in closed chambers. A calculated concentration of six milligrams of BPL per liter of air is needed at the beginning of the contact period, followed one-half hour later by three milligrams of BPL per liter of air for a total contact time of one and one-half hours to complete the sterilization. A device that rapidly disseminates the BPL-water mixture should be used so that a sterilizing concentration is maintained during the entire contact time.

The quantity of residual beta-propiolactone present one-half hour after commencing the water wash following sterilization is so negligible that tests capable of detecting one gamma per liter of air did not indicate the presence of beta-propiolactone. Tests indicated, moreover, that no neutralization of BPL or its breakdown products was needed.

Neoprene products deteriorate slightly following exposure to beta-propiolactone. This effect may not be detected for months in the case of thick neoprene valve diaphragms, etc., but gloves may become tacky after a single application. Tackiness may be avoided by washing the neoprene product with water or a 0.5 per cent solution of sodium thiosulfate immediately after contact is terminated.

Copper or brass products may be oxidized slightly by beta-propiolactone but not to a serious extent. Stainless steel is not affected, although galvanic corrosion may be accelerated if it is in contact with dissimilar metals.

The sterilization efficacy of BPL vapor is a direct function of the concentration, relative humidity, and temperature. A temperature of 25°C. or higher, and a relative humidity between 70 and 85 per cent have proved satisfactory.

Particular attention should be given to recessed areas within the chamber to be sterilized, to assure that all areas can be reached by the beta-propiolactone vapor.

BPL vapor has several advantages over formaldehyde as a sterilant:

(a) Its vapors are lachrymatory, giving BPL its own warning device, but are less irritating than those of formaldehyde.

(b) It does not polymerize as readily as formaldehyde.

(c) It is readily soluble in water.

(d) In aqueous solution, it has a short half-life, which may be reduced further by increasing the temperature.

(e) It is easily removed by air washing.

(f) It acts more rapidly than formaldehyde; however, in the liquid state BPL is more toxic than formaldehyde.

Acknowledgements

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Appendix A

Laboratory Assay Procedures for Beta-Propiolactone

Two basic laboratory procedures are used in assaying BPL. The first procedure (deterioration detection) is used for assay of the bulk product; the second procedure (detection of residual BPL) is used to assay samples containing low concentrations of BPL.

Deterioration Detection

The concentration of BPL in bulk must be determined immediately upon receipt and periodically during storage to indicate possible deterioration during transit and storage.

1. Pipette 25 milliliters of 0.4 molar sodium thiosulfate into a flask and add 1 milliliter of 2 M dibasic potassium phosphate buffer solution.

2. Weigh out a sample of BPL in a small glass vial, between 0.3 and 0.4 gram, known to the nearest 0.1 milligram.

3. Place the weighed vial containing the BPL in the flask containing the thiosulfate-buffer solution.

4. Swirl gently to mix and allow to react for 10 to 20 minutes.

5. After this reaction period, wash down the sides of the flask with about 25 milliliters of water and titrate with 0.2 N iodine solution, using starch as an indicator.

6. Blanks: All blanks should be run so as to simulate run conditions to correct for minor errors that may occur when the thiosulfate is titrated in weakly alkaline solution. Add 5 milliliters of sodium acetate before titration.

$$\text{Per cent beta-propiolactone} = \frac{(A - B)\ N \times 7.206}{Wt \times 0.995}$$

where

A is milliliter of iodine required for blank,

B is milliliter of iodine required for run,

N is normality of iodine, and

Wt is weight of sample in milligrams

The correction factor, 0.995, is based upon results obtained by cryoscopic analysis and represents the practical product purity.

7. Reagents. Sodium thiosulfate approximately 0.4 Molar

Standard iodine solution 0.2 Normal

Dibasic potassium phosphate buffer solution

(K_2HPO_4)	2 Molar
Sodium acetate	1 Molar
Starch solution	0.5 per cent

8. The reaction involved in the determination is

$$\begin{array}{l} CH_2-CH_2 \\ O\text{——}C=O \end{array} \times$$

$$Na_2S_2O_3 \ - \ NaO_3S\text{-}SCH_2CH_2COONa$$

9. The above method of analysis for beta-propiolactone was excerpted from "Chemical and Cryoscopic Analysis of B-Propiolactone" by Willard P. Tyler and Donald W. Beesing, *Analytical Chemistry*, Vol. 24, Page 1511, Sept. 1952.

Detection of Residual BPL

The BPL in air is assayed by the following procedures:

1. Reagents

20 per cent sodium hydroxide (NaOH)

20 per cent hydroxylamine hydrochloride (NH_2OH-HCl)

$FeCl_3$ ($6H_2O$) 10 per cent by weight in 6N hydrochloric acid (HCl)

2. Procedure

a. Immediately prior to sampling, place 2.5 milliliter each of NaOH and NH_2OH-HCl solutions in the gas washing bottle (Dreschsel) for collecting the aerosol sample.

b. Draw a measured volume of air containing BPL through the bubbler.

c. Add three milliliters of $FeCl_3$ reagent.

d. Shake bubbler to remove entrapped air.

e. Dilute to 100 milliliter with distilled water.

f. If a greater volume of reagents is required because of high BPL content of the sample, add proportionately more reagents, i.e., 2.5 milliliters NaOH plus 2.4 milliliters NH_2OH-HCl plus 3 milliliters $FeCl_3$.

g. Check samples of the test solution for light transmission in an electrophotometer. Compare with a reagent blank that does not contain BPL. A blank is prepared with five milliliters of the NaOH solution, five milliliters of the NH_2OH-HCl solution, and six milliliters $FeCl_3$ solution. The mixture is brought to 100 milliliters with distilled water. The blank is used to obtain a zero reading after the electrophotometer is nulled. A 525-millimicron filter is used for all determinations.

h. The value obtained by deducting the reading of the blank from the reading of the test solution can then be compared with a standard curve to obtain the milligrams of BPL contained in the sample.

Appendix B

Disseminating Devices

The sources of BPL disseminating devices found to be satisfactory listed below represent only those types tested or used at Fort Detrick. This list probably does not include others commercially available, and is not to be construed as an endorsement of the manufacturer's product.

Pulse Jet M3A3 Smoke Generator
Army Chemical Center
Edgewood, Md.

Hydromist Vaporizer
Arnold Laboratories
2507 S. Main St.
Los Angeles

Tokheim Vaporizer
1672 Wabash Ave.
Ft. Wayne, Ind.

Todd Insecticidal Fog Applicator
Army Chemical Center
Edgewood, Md.

Challenger Vaporizer
Z & W Jet Sprayer Model 5100C
Z & W Manufacturing Corp.
30240 Lakelaid Blvd.
Wickliffe, O.